

A robust ambient temperature collection and stabilization strategy: Enabling worldwide functional studies of the human microbiome

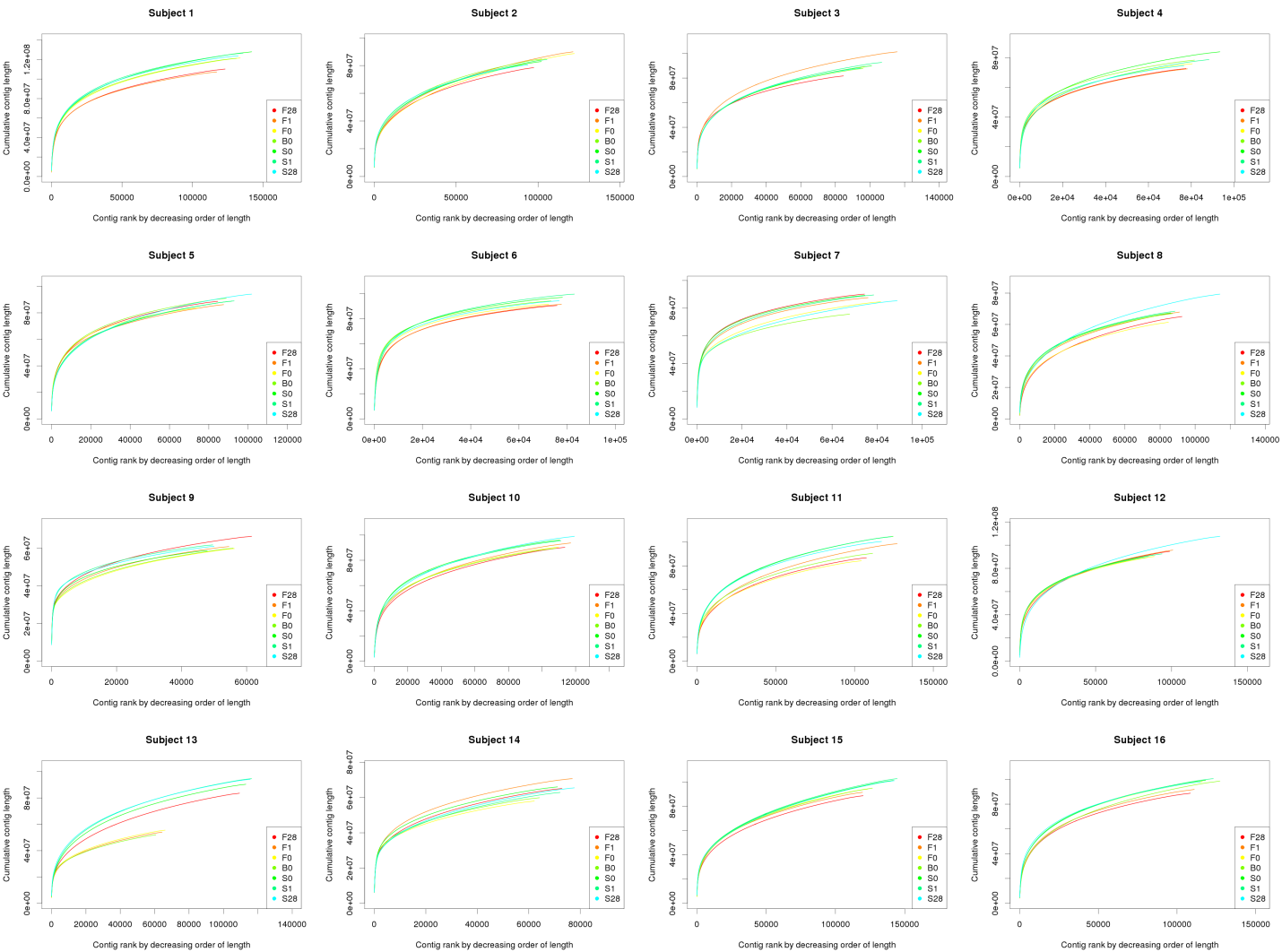
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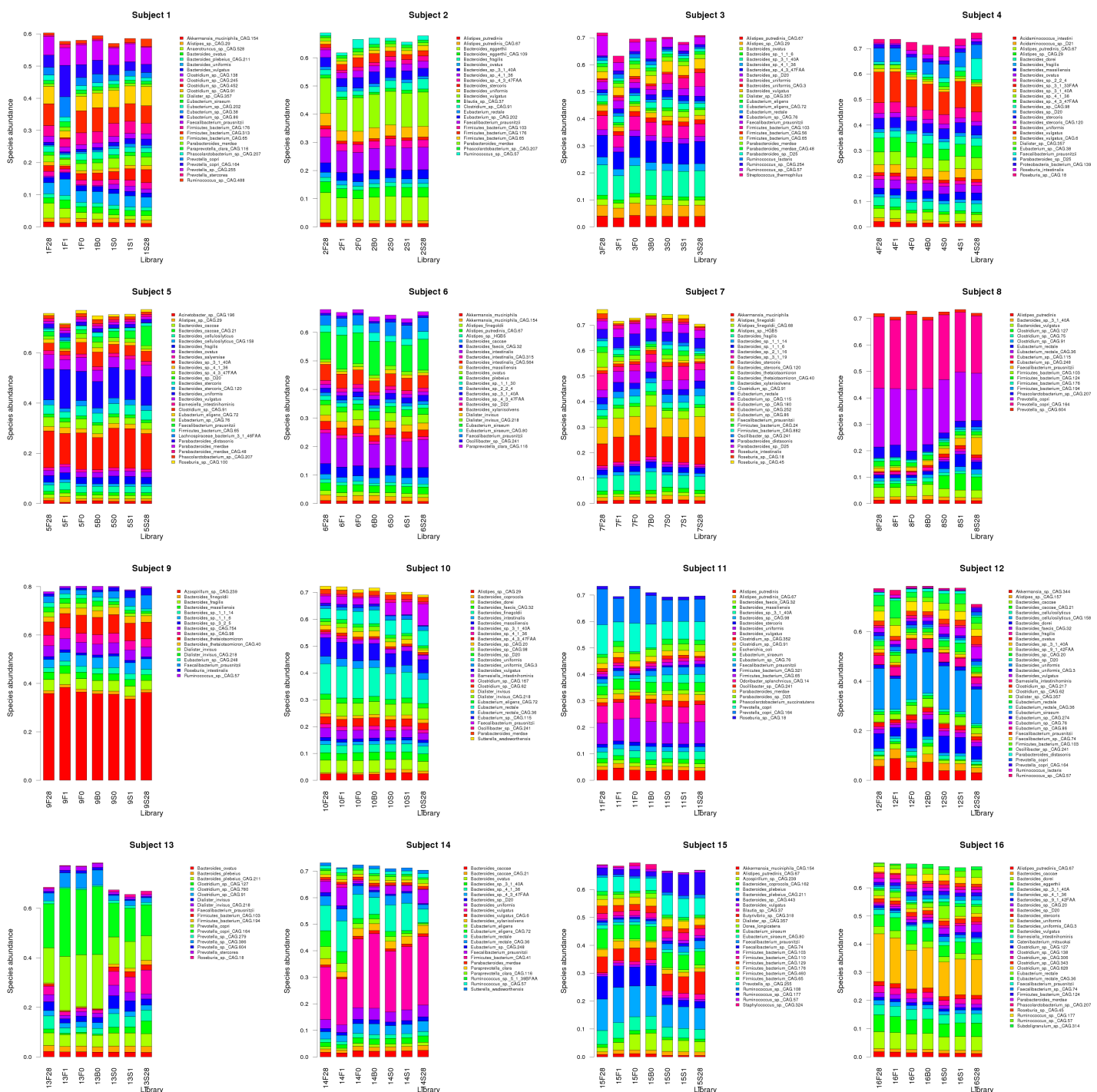
² Genomic Medicine, J. Craig Venter Institute, La Jolla, CA 92037.

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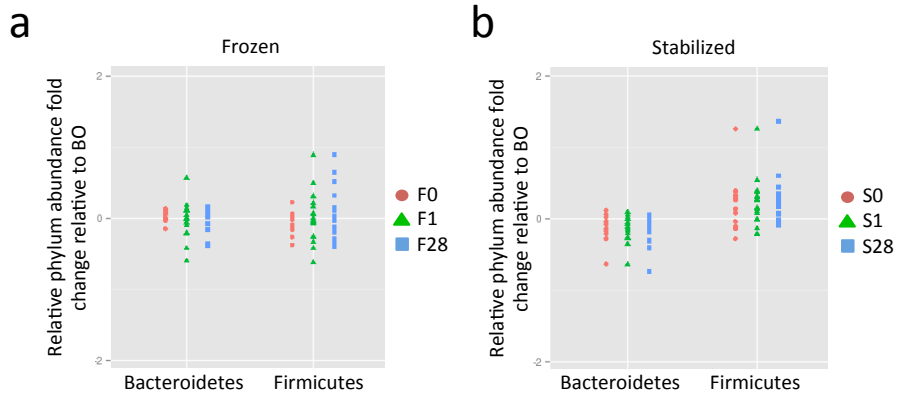
Supplemental Information



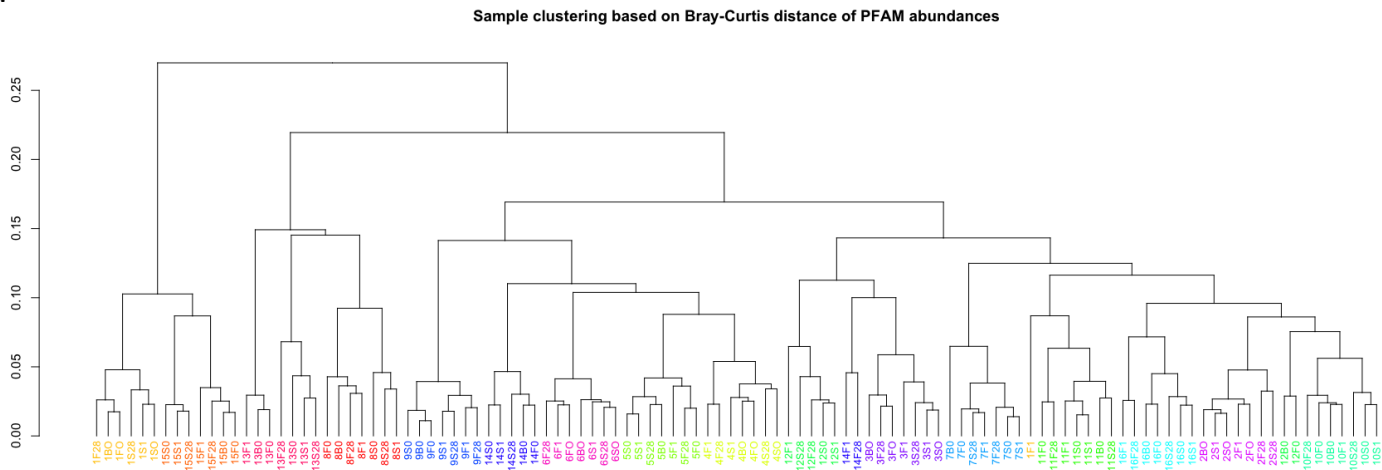
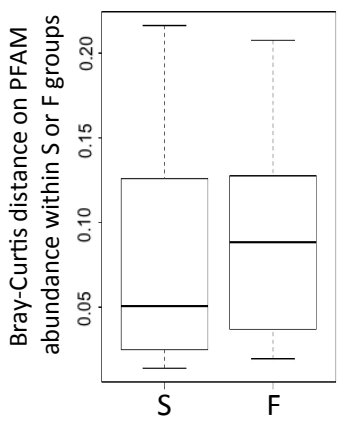
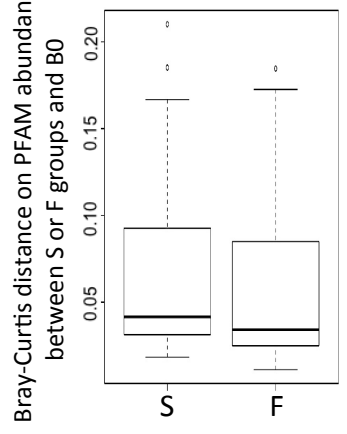
Supplemental Figure S1. Assembly Metrics are unaffected by stabilization method. Total contig length is plotted against contig rank in the order of decreasing length for all individual subjects. B0: Basal; F0, F1, F28: Frozen Day 0, Day 1, and Day 28; S0, S1, S28: Stabilized Day 0, Day 1, and Day 28.



Supplemental Figure S2. Relative species abundance of individual subjects. Species abundance is plotted for Frozen, Basal, and Stabilized samples from each individual subject. B0: Basal; F0, F1, F28: Frozen Day 0, Day 1, and Day 28; S0, S1, S28: Stabilized Day 0, Day 1, and Day 28.



Supplemental Figure S3. Bacteroidetes and Firmicutes phyla are unchanged relative to Basal in Frozen and Stabilized samples. Fold change in Bacteroidetes or Firmicutes phyla relative to Basal across the time course in (a) Frozen or (b) Stabilized samples. F0, F1, F28: Frozen Day 0, Day 1, and Day 28; S0, S1, S28: Stabilized Day 0, Day 1, and Day 28.

a**b****c**

Supplemental Figure S4. Stabilized samples maintain results of Basal and Frozen Pfam analysis. (a) Bray-Curtis dissimilarity based on relative Pfam abundances were used to cluster all samples. Each color represents a distinct subject. (b) Box and whisker plots of calculated pairwise Bray-Curtis dissimilarity within stabilization method. (c) Box and whisker plots of calculated pairwise Bray-Curtis dissimilarity between Stabilized or Frozen samples and Basal. B0: Basal; F0, F1, F28: Frozen Day 0, Day 1, and Day 28; S0, S1, S28: Stabilized Day 0, Day 1, and Day 28; F: Frozen; S: Stabilized.

Supplemental Table S1. Sequencing metrics from RNA seq on a Stabilized stool sample		
Sample	Stabilized RNA1A	Stabilized RNA1B
% rRNA	0.0165	0.0665
total rRNA	92743	255013
# 23S reads	62656	174727
# 16S reads	28573	79021
# 5S reads	1514	1265
# unmapped reads	807651	589168
# Viruses reads	656	859
# Eukaryota reads	903	1341
# Archaea reads	83	56
# Bacteria reads	4824571	3240490
# mapped reads	4826194	3242791
# non-human reads (pair)	5633845	3831959
# human reads (pair)	9168	13895
# of Low quality reads	0.2722	0.361
# good reads (pair)	5643013	3845854
Raw reads (GB)	1.938483	1.50487
# raw reads (pair)	7753933	6019481

Sequencing metrics from an RNASeq Stabilized sample.

Sequencing metrics were tabulated for sequencing libraries made from two replicate RNA extractions from a single Stabilized sample.

Supplemental Table S2. Individual Sample QC and sequencing metrics

Sample	Number passed reads (pair)	Fraction of Low quality reads	% Duplicated reads	Number mapped reads	Number unmapped reads	Number of non-human reads (pair)	% human reads (pair)	% Bacteria reads	% Archaea reads	% Eukaryota reads	% Virus reads	Total contig length (bp)	Contig N50 (bp)	Longest contig (bp)
1B0	1.7E+07	0.1517	15.87	1.3E+07	4.0E+06	1.7E+07	0.26	76.15	0.154	0.04	0.007	2.09E+08	4280	5.72E+05
2B0	2.3E+07	0.147	9.50	1.9E+07	3.9E+06	2.3E+07	0.04	82.60	0.002	0.00	0.007	1.91E+08	4073	3.27E+05
3B0	2.7E+07	0.2004	11.72	2.4E+07	3.7E+06	2.7E+07	0.01	86.64	0.002	0.01	0.001	1.96E+08	3630	3.61E+05
4B0	2.3E+07	0.1594	9.61	2.2E+07	1.9E+06	2.3E+07	0.05	91.72	0.000	0.00	0.002	1.51E+08	3382	8.13E+05
5B0	2.4E+07	0.1856	10.63	2.1E+07	3.0E+06	2.4E+07	0.01	87.32	0.001	0.00	0.001	1.70E+08	5651	4.44E+05
6B0	1.7E+07	0.1588	11.29	1.5E+07	2.4E+06	1.7E+07	0.02	86.19	0.020	0.00	0.007	1.38E+08	11349	4.10E+05
7B0	2.2E+07	0.1645	10.43	1.9E+07	3.1E+06	2.2E+07	0.08	85.76	0.001	0.00	0.002	1.34E+08	8773	1.09E+06
8B0	1.9E+07	0.1682	9.97	1.4E+07	4.6E+06	1.9E+07	0.03	75.16	0.025	0.00	0.003	1.42E+08	2191	2.54E+05
9B0	2.5E+07	0.2145	12.92	2.1E+07	3.9E+06	2.5E+07	0.07	84.06	0.001	0.00	0.000	1.17E+08	4527	8.02E+05
10B0	2.3E+07	0.189	10.22	2.0E+07	3.2E+06	2.3E+07	0.02	86.20	0.001	0.05	0.001	1.80E+08	2580	4.74E+05
11B0	2.1E+07	0.2239	15.56	1.7E+07	4.1E+06	2.1E+07	0.09	80.11	0.001	0.01	0.002	1.70E+08	2654	3.28E+05
12B0	1.9E+07	0.2228	10.28	1.7E+07	2.5E+06	1.9E+07	0.06	86.91	0.001	0.00	0.000	1.49E+08	4770	4.13E+05
13B0	2.1E+07	0.2581	11.28	1.6E+07	5.2E+06	2.1E+07	0.12	74.70	0.002	0.01	0.000	1.11E+08	1860	3.07E+05
14B0	2.2E+07	0.2194	8.86	1.9E+07	3.1E+06	2.2E+07	0.03	85.66	0.000	0.01	0.006	1.20E+08	2457	4.65E+05
15B0	2.7E+07	0.2129	9.32	2.0E+07	6.4E+06	2.7E+07	0.07	75.59	0.076	0.03	0.007	2.53E+08	1438	4.16E+05
16B0	2.3E+07	0.2159	11.44	1.9E+07	4.1E+06	2.3E+07	0.02	82.22	0.121	0.01	0.003	1.92E+08	3450	6.28E+05
1F0	1.7E+07	0.1577	17.52	1.3E+07	4.1E+06	1.7E+07	0.28	75.77	0.153	0.04	0.007	2.10E+08	3991	4.61E+05
2F0	3.2E+07	0.2187	11.16	2.6E+07	5.7E+06	3.2E+07	0.11	82.24	0.002	0.00	0.007	2.10E+08	3991	4.61E+05
3F0	2.7E+07	0.2046	11.95	2.4E+07	3.4E+06	2.7E+07	0.01	87.33	0.002	0.01	0.001	2.39E+08	2923	3.04E+05
4F0	2.4E+07	0.1673	10.29	2.2E+07	1.9E+06	2.4E+07	0.03	92.13	0.000	0.00	0.002	1.91E+08	4055	4.66E+05
5F0	2.1E+07	0.1714	11.67	1.8E+07	2.3E+06	2.1E+07	0.02	89.04	0.001	0.00	0.001	1.47E+08	3131	8.18E+05
6F0	1.7E+07	0.1705	13.38	1.5E+07	2.3E+06	1.7E+07	0.04	86.89	0.020	0.00	0.005	1.48E+08	6546	4.44E+05
7F0	2.5E+07	0.1786	9.93	2.1E+07	3.8E+06	2.5E+07	0.07	84.41	0.001	0.00	0.002	1.30E+08	12205	4.09E+05
8F0	1.9E+07	0.1917	12.97	1.5E+07	4.5E+06	1.9E+07	0.03	76.15	0.014	0.00	0.002	1.56E+08	8306	8.94E+05
9F0	2.5E+07	0.2031	9.50	2.1E+07	3.8E+06	2.5E+07	0.07	84.40	0.001	0.00	0.000	1.28E+08	2393	2.18E+05
10F0	2.4E+07	0.1865	10.21	2.1E+07	3.2E+06	2.4E+07	0.03	86.72	0.001	0.05	0.001	1.17E+08	6024	8.01E+05
11F0	2.2E+07	0.2278	17.76	1.8E+07	4.1E+06	2.2E+07	0.05	81.51	0.001	0.01	0.001	1.85E+08	2823	5.48E+05
12F0	2.1E+07	0.2453	10.58	1.8E+07	2.6E+06	2.1E+07	0.03	87.39	0.001	0.00	0.000	1.59E+08	2996	3.28E+05

Supplemental Table S2 cont'd

13F0	2.0E+07	0.2643	11.31	1.5E+07	5.0E+06	2.0E+07	0.03	75.17	0.002	0.00	0.000	1.54E+08	4684	3.91E+05
14F0	2.1E+07	0.2129	8.84	1.8E+07	2.7E+06	2.1E+07	0.02	86.88	0.001	0.01	0.006	1.13E+08	2075	3.07E+05
15F0	2.4E+07	0.2081	8.34	1.8E+07	5.8E+06	2.4E+07	0.07	75.99	0.060	0.02	0.007	1.11E+08	2790	4.65E+05
16F0	1.9E+07	0.191	10.54	1.6E+07	3.5E+06	1.9E+07	0.03	81.73	0.135	0.01	0.005	2.35E+08	1605	3.83E+05
1F1	1.5E+07	0.1733	24.34	1.3E+07	2.9E+06	1.5E+07	0.23	80.39	0.499	0.01	0.002	1.67E+08	3794	3.98E+05
2F1	2.1E+07	0.173	14.65	1.7E+07	3.6E+06	2.1E+07	0.03	82.56	0.002	0.01	0.010	1.90E+08	3364	3.46E+05
3F1	2.0E+07	0.1842	14.40	1.7E+07	2.9E+06	2.0E+07	0.02	85.09	0.002	0.01	0.002	1.95E+08	3944	4.17E+05
4F1	2.1E+07	0.1721	9.64	1.9E+07	1.6E+06	2.1E+07	0.02	92.33	0.000	0.00	0.001	1.33E+08	2889	8.13E+05
5F1	2.1E+07	0.2226	12.71	1.8E+07	3.1E+06	2.1E+07	0.02	85.32	0.001	0.00	0.003	1.64E+08	3911	4.73E+05
6F1	1.8E+07	0.1906	16.11	1.5E+07	2.3E+06	1.8E+07	0.07	87.05	0.041	0.00	0.008	1.31E+08	10404	4.09E+05
7F1	2.0E+07	0.1637	12.48	1.7E+07	3.1E+06	2.0E+07	0.05	84.40	0.001	0.00	0.002	1.42E+08	12646	8.94E+05
8F1	2.1E+07	0.1979	12.78	1.6E+07	4.9E+06	2.1E+07	0.06	76.42	0.100	0.00	0.003	1.49E+08	2357	3.24E+05
9F1	1.9E+07	0.1993	8.09	1.6E+07	3.2E+06	1.9E+07	0.15	83.36	0.001	0.00	0.000	1.07E+08	5507	5.73E+05
10F1	2.9E+07	0.2074	11.88	2.5E+07	4.1E+06	2.9E+07	0.02	85.92	0.001	0.04	0.001	2.01E+08	2828	5.47E+05
11F1	2.3E+07	0.2821	14.69	1.8E+07	4.4E+06	2.3E+07	0.10	80.79	0.002	0.01	0.002	1.92E+08	2637	3.63E+05
12F1	1.8E+07	0.2687	14.66	1.5E+07	3.1E+06	1.8E+07	0.04	82.82	0.002	0.00	0.000	1.55E+08	4169	4.24E+05
13F1	7.3E+06	0.2563	7.00	5.4E+06	1.8E+06	7.3E+06	0.03	74.95	0.002	0.01	0.000	5.69E+07	1560	2.24E+05
14F1	2.4E+07	0.2817	9.61	2.0E+07	3.7E+06	2.3E+07	0.03	84.22	0.001	0.01	0.005	1.44E+08	2017	4.64E+05
15F1	2.2E+07	0.1956	8.80	1.7E+07	5.0E+06	2.2E+07	0.06	76.82	0.190	0.02	0.006	2.16E+08	1550	3.73E+05
16F1	1.8E+07	0.196	13.21	1.5E+07	3.0E+06	1.8E+07	0.02	83.52	0.308	0.01	0.004	1.57E+08	3459	2.89E+05
1F28	1.4E+07	0.1694	26.87	1.1E+07	2.8E+06	1.4E+07	0.11	79.60	0.066	0.01	0.001	1.45E+08	2792	4.51E+05
2F28	2.3E+07	0.1955	18.23	1.9E+07	4.6E+06	2.3E+07	0.03	79.96	0.003	0.01	0.013	1.69E+08	3224	2.71E+05
3F28	2.5E+07	0.2418	17.85	2.1E+07	3.9E+06	2.5E+07	0.02	84.15	0.002	0.01	0.002	1.63E+08	3831	4.22E+05
4F28	2.8E+07	0.2299	11.54	2.5E+07	2.9E+06	2.8E+07	0.01	89.58	0.001	0.00	0.003	1.42E+08	2939	8.18E+05
5F28	2.6E+07	0.299	14.14	2.3E+07	3.8E+06	2.6E+07	0.01	85.76	0.002	0.00	0.001	1.56E+08	6474	4.16E+05
6F28	2.2E+07	0.2164	13.06	1.7E+07	5.1E+06	2.2E+07	0.05	76.72	0.337	0.00	0.019	1.40E+08	13134	4.40E+05
7F28	2.4E+07	0.2753	10.59	1.8E+07	5.8E+06	2.4E+07	0.12	75.61	0.003	0.01	0.007	1.49E+08	8002	1.09E+06
8F28	2.3E+07	0.267	10.63	1.7E+07	5.7E+06	2.3E+07	0.05	74.79	0.480	0.01	0.009	1.64E+08	1576	2.77E+05
9F28	2.4E+07	0.2435	10.30	2.0E+07	4.4E+06	2.4E+07	0.08	81.51	0.002	0.00	0.000	1.26E+08	4557	8.02E+05
10F28	2.2E+07	0.3223	11.62	1.7E+07	4.5E+06	2.2E+07	0.05	79.37	0.003	0.24	0.005	1.85E+08	2023	5.38E+05
11F28	1.8E+07	0.3082	30.59	1.3E+07	4.6E+06	1.8E+07	0.24	74.51	0.003	0.01	0.004	1.42E+08	3104	4.36E+05
12F28	1.6E+07	0.2724	13.66	1.3E+07	3.5E+06	1.6E+07	0.07	78.75	0.002	0.01	0.000	1.56E+08	4197	3.91E+05

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13F28	2.2E+07	0.3375	13.53	1.6E+07	5.2E+06	2.2E+07	0.11	75.93	0.003	0.01	0.001	1.59E+08	2740	2.54E+05
14F28	2.0E+07	0.3413	12.04	1.6E+07	4.0E+06	2.0E+07	0.03	80.07	0.001	0.02	0.005	1.20E+08	2730	4.65E+05
15F28	2.2E+07	0.2542	9.01	1.5E+07	6.7E+06	2.2E+07	0.08	67.92	1.523	0.01	0.009	2.19E+08	1396	4.38E+05
16F28	1.6E+07	0.2201	17.99	1.3E+07	3.2E+06	1.6E+07	0.03	78.64	1.772	0.01	0.007	1.38E+08	2988	3.33E+05
1S0	1.5E+07	0.1442	13.79	1.2E+07	3.8E+06	1.5E+07	0.10	75.13	0.135	0.02	0.005	2.10E+08	4170	6.07E+05
2S0	2.0E+07	0.1542	10.95	1.6E+07	3.5E+06	2.0E+07	0.01	82.43	0.002	0.00	0.007	1.76E+08	3455	2.92E+05
3S0	2.8E+07	0.2153	10.84	2.4E+07	3.9E+06	2.8E+07	0.01	86.22	0.002	0.00	0.001	2.10E+08	2876	3.62E+05
4S0	2.9E+07	0.1718	9.34	2.6E+07	2.4E+06	2.9E+07	0.01	91.58	0.001	0.00	0.002	1.71E+08	3865	8.18E+05
5S0	2.7E+07	0.1806	11.40	2.4E+07	3.1E+06	2.7E+07	0.01	88.55	0.001	0.00	0.000	1.88E+08	3957	5.01E+05
6S0	1.6E+07	0.162	11.11	1.4E+07	2.2E+06	1.6E+07	0.05	86.85	0.012	0.00	0.004	1.36E+08	10756	3.38E+05
7S0	2.3E+07	0.1712	9.38	1.9E+07	3.8E+06	2.3E+07	0.01	83.69	0.002	0.00	0.001	1.58E+08	8139	1.09E+06
8S0	1.9E+07	0.1757	9.43	1.4E+07	4.4E+06	1.9E+07	0.03	76.43	0.015	0.00	0.002	1.41E+08	2039	3.24E+05
9S0	2.4E+07	0.1834	8.92	2.1E+07	3.7E+06	2.4E+07	0.08	84.44	0.001	0.00	0.000	1.11E+08	5678	8.02E+05
10S0	2.1E+07	0.1723	9.56	1.8E+07	3.1E+06	2.1E+07	0.03	85.16	0.002	0.02	0.001	1.80E+08	2864	5.38E+05
11S0	2.2E+07	0.2429	12.13	1.8E+07	4.0E+06	2.2E+07	0.11	81.71	0.002	0.01	0.001	1.92E+08	3168	3.56E+05
12S0	1.8E+07	0.2314	8.79	1.6E+07	2.7E+06	1.8E+07	0.07	85.08	0.002	0.01	0.000	1.52E+08	4315	4.23E+05
13S0	6.8E+06	0.2731	6.05	5.0E+06	1.8E+06	6.8E+06	0.03	74.02	0.002	0.01	0.000	9.04E+07	1240	3.07E+05
14S0	2.1E+07	0.2131	8.24	1.8E+07	3.1E+06	2.1E+07	0.02	85.24	0.001	0.01	0.004	1.28E+08	2629	4.65E+05
15S0	2.2E+07	0.1952	7.79	1.7E+07	5.4E+06	2.2E+07	0.03	75.50	0.043	0.01	0.005	2.46E+08	1647	3.86E+05
16S0	1.9E+07	0.1899	9.82	1.6E+07	3.4E+06	1.9E+07	0.02	82.03	0.099	0.01	0.003	1.81E+08	3451	4.50E+05
1S1	1.2E+07	0.1385	12.59	9.2E+06	2.9E+06	1.2E+07	0.09	75.85	0.098	0.02	0.005	1.81E+08	3932	4.91E+05
2S1	1.8E+07	0.1524	10.25	1.5E+07	3.0E+06	1.8E+07	0.01	82.79	0.002	0.00	0.007	1.69E+08	2928	2.94E+05
3S1	2.6E+07	0.198	10.80	2.3E+07	3.5E+06	2.6E+07	0.01	86.35	0.002	0.01	0.001	2.10E+08	3061	3.62E+05
4S1	2.9E+07	0.1857	8.97	2.7E+07	2.2E+06	2.9E+07	0.01	92.46	0.001	0.00	0.001	1.61E+08	3212	7.37E+05
5S1	2.7E+07	0.187	11.01	2.4E+07	3.1E+06	2.7E+07	0.01	88.50	0.001	0.00	0.000	1.90E+08	3597	4.31E+05
6S1	2.1E+07	0.1708	10.15	1.8E+07	2.8E+06	2.1E+07	0.05	86.36	0.019	0.00	0.006	1.52E+08	11826	3.38E+05
7S1	2.3E+07	0.1714	9.03	1.9E+07	3.5E+06	2.3E+07	0.01	84.31	0.002	0.00	0.001	1.58E+08	7339	1.09E+06
8S1	1.8E+07	0.1738	9.33	1.4E+07	4.2E+06	1.8E+07	0.03	77.32	0.011	0.00	0.002	1.43E+08	1995	3.24E+05
9S1	2.3E+07	0.1812	8.39	1.9E+07	3.5E+06	2.3E+07	0.08	84.34	0.001	0.00	0.000	1.12E+08	5251	8.03E+05
10S1	2.4E+07	0.1686	9.57	2.0E+07	3.5E+06	2.4E+07	0.03	85.28	0.002	0.02	0.000	1.94E+08	2789	6.62E+05
11S1	2.0E+07	0.2246	12.57	1.6E+07	3.7E+06	2.0E+07	0.11	81.34	0.001	0.01	0.001	1.84E+08	3081	3.28E+05
12S1	2.0E+07	0.2254	9.11	1.7E+07	3.0E+06	2.0E+07	0.03	84.99	0.002	0.00	0.000	1.62E+08	3965	3.90E+05

Supplemental Table S2 cont'd

13S1	2.0E+07	0.2549	10.98	1.5E+07	5.3E+06	2.0E+07	0.03	74.15	0.002	0.01	0.000	1.81E+08	2728	4.92E+05
14S1	2.1E+07	0.2205	8.33	1.8E+07	2.8E+06	2.1E+07	0.02	86.65	0.001	0.01	0.004	1.28E+08	2098	4.65E+05
15S1	2.1E+07	0.1952	7.82	1.6E+07	5.1E+06	2.1E+07	0.03	75.50	0.041	0.01	0.004	2.40E+08	1610	3.86E+05
16S1	1.9E+07	0.1819	9.65	1.6E+07	3.4E+06	1.9E+07	0.02	82.00	0.074	0.01	0.003	1.83E+08	3499	5.32E+05
1S28	8.6E+06	0.1372	12.91	6.6E+06	2.0E+06	8.6E+06	0.12	76.25	0.062	0.02	0.005	1.46E+08	2757	7.23E+05
2S28	1.2E+07	0.1412	8.84	9.6E+06	2.1E+06	1.2E+07	0.02	82.40	0.002	0.01	0.005	1.21E+08	2735	2.85E+05
3S28	2.4E+07	0.1934	11.93	2.1E+07	3.1E+06	2.4E+07	0.01	86.87	0.002	0.01	0.001	1.88E+08	3033	3.56E+05
4S28	2.7E+07	0.1732	9.82	2.5E+07	2.1E+06	2.7E+07	0.01	92.36	0.000	0.00	0.001	1.47E+08	3549	8.13E+05
5S28	2.5E+07	0.2105	9.27	2.2E+07	2.9E+06	2.5E+07	0.01	88.67	0.001	0.00	0.000	1.77E+08	4192	3.60E+05
6S28	1.6E+07	0.1607	10.79	1.4E+07	2.1E+06	1.6E+07	0.05	87.20	0.009	0.00	0.003	1.33E+08	10284	4.09E+05
7S28	2.5E+07	0.236	11.43	2.1E+07	3.8E+06	2.5E+07	0.04	84.80	0.001	0.00	0.001	1.59E+08	5468	1.09E+06
8S28	2.0E+07	0.1867	8.62	1.5E+07	4.7E+06	2.0E+07	0.03	76.69	0.017	0.00	0.002	1.70E+08	1564	3.24E+05
9S28	2.3E+07	0.203	8.43	2.0E+07	3.5E+06	2.3E+07	0.07	84.89	0.001	0.00	0.000	1.11E+08	5656	6.21E+05
10S28	2.2E+07	0.1936	8.69	1.9E+07	3.3E+06	2.2E+07	0.03	84.72	0.002	0.02	0.001	1.94E+08	2483	4.74E+05
11S28	1.5E+07	0.2154	10.64	1.2E+07	2.7E+06	1.5E+07	0.09	81.81	0.001	0.01	0.001	1.57E+08	2855	3.28E+05
12S28	1.6E+07	0.2223	7.97	1.4E+07	2.6E+06	1.6E+07	0.06	84.26	0.001	0.00	0.000	1.82E+08	2063	3.83E+05
13S28	1.9E+07	0.2697	8.99	1.4E+07	4.7E+06	1.9E+07	0.03	75.30	0.002	0.01	0.000	1.76E+08	2477	5.28E+05
14S28	2.0E+07	0.2212	8.47	1.8E+07	2.5E+06	2.0E+07	0.02	87.68	0.000	0.01	0.004	1.31E+08	1981	4.65E+05
15S28	2.2E+07	0.2004	7.61	1.7E+07	5.1E+06	2.2E+07	0.03	76.63	0.027	0.01	0.004	2.42E+08	1635	3.87E+05
16S28	1.5E+07	0.1871	10.18	1.2E+07	2.6E+06	1.5E+07	0.02	81.74	0.086	0.01	0.004	1.56E+08	3270	3.98E+05

Individual Sample Sequencing Metrics. Sequencing metrics were tabulated for each sample. Averaged sequencing metrics are shown in Table 1.

Supplementary Table S3. Sequencing assembly metrics

Sample	Treatment	Subject	Number non-human reads for assembly (pair)	Total contig length (bp)	Contig N50 (bp)	Longest contig (bp)
1B0	B0	1	6,747,334	121,352,726	2,189	110,081
2B0	B0	2	6,762,180	84,771,234	1,398	451,141
3B0	B0	3	6,764,056	87,833,571	1,903	251,829
4B0	B0	4	6,762,066	78,448,068	2,768	185,182
5B0	B0	5	6,764,199	90,952,224	2,450	272,345
6B0	B0	6	6,763,544	94,205,222	6,952	210,048
7B0	B0	7	6,759,682	75,589,632	6,073	236,758
8B0	B0	8	6,762,882	66,958,700	1,515	364,716
9B0	B0	9	6,760,203	60,027,286	3,836	361,176
10B0	B0	10	6,763,757	89,549,999	1,756	215,299
11B0	B0	11	6,758,786	90,521,351	1,370	230,787
12B0	B0	12	6,761,243	89,549,600	2,599	342,996
13B0	B0	13	6,756,597	51,930,157	1,891	359,668
14B0	B0	14	6,763,289	59,863,002	2,981	363,194
15B0	B0	15	6,760,612	94,799,537	1,164	469,211
16B0	B0	16	6,763,952	98,503,972	1,246	134,392
1F0	F0	1	6,745,916	121,687,862	2,074	117,508
2F0	F0	2	6,757,493	88,704,376	987	434,010
3F0	F0	3	6,764,309	88,990,759	1,886	217,752
4F0	F0	4	6,763,109	76,017,652	2,486	223,525
5F0	F0	5	6,763,983	83,704,561	2,946	277,815
6F0	F0	6	6,762,479	91,621,948	6,195	339,338
7F0	F0	7	6,760,357	84,575,506	4,044	206,096
8F0	F0	8	6,762,917	61,421,024	1,215	235,064
9F0	F0	9	6,760,330	59,830,297	3,703	360,105
10F0	F0	10	6,763,186	90,549,075	1,728	469,082
11F0	F0	11	6,761,435	84,335,355	1,324	132,083
12F0	F0	12	6,763,038	90,244,109	2,382	426,787
13F0	F0	13	6,762,856	55,517,002	1,601	148,812
14F0	F0	14	6,763,572	57,957,367	3,465	434,374
15F0	F0	15	6,760,230	94,116,652	1,249	337,881
16F0	F0	16	6,762,880	98,099,413	1,589	185,427
1F1	F1	1	6,749,170	107,359,637	2,331	339,812
2F1	F1	2	6,763,158	89,929,438	1,076	188,424
3F1	F1	3	6,763,997	101,358,262	1,693	381,510
4F1	F1	4	6,763,565	72,528,185	2,511	422,394
5F1	F1	5	6,763,687	86,141,906	2,498	230,323
6F1	F1	6	6,760,648	91,515,702	4,430	257,025
7F1	F1	7	6,762,012	87,558,094	4,533	551,204
8F1	F1	8	6,760,674	67,893,081	1,318	360,598
9F1	F1	9	6,754,942	60,872,436	4,433	343,714
10F1	F1	10	6,763,436	93,742,679	1,471	492,536
11F1	F1	11	6,758,394	98,748,518	1,218	147,992
12F1	F1	12	6,762,549	95,703,213	1,883	569,101

Supplemental Table S3 cont'd

13F1	F1	13	6,762,797	53,968,796	1,634	252,023
14F1	F1	14	6,763,292	70,708,404	2,179	272,631
15F1	F1	15	6,761,152	91,424,771	1,298	239,217
16F1	F1	16	6,763,626	91,778,142	1,535	306,862
1F28	F28	1	6,747,041	110,265,992	2,084	384,451
2F28	F28	2	6,763,805	78,515,157	1,349	201,984
3F28	F28	3	6,764,249	81,750,725	2,511	307,209
4F28	F28	4	6,763,792	72,750,082	2,515	464,327
5F28	F28	5	6,764,222	88,617,977	2,488	339,772
6F28	F28	6	6,762,192	90,549,814	4,752	570,885
7F28	F28	7	6,764,130	90,120,282	5,569	356,533
8F28	F28	8	6,762,965	65,060,741	1,079	563,453
9F28	F28	9	6,734,112	66,329,318	3,148	163,253
10F28	F28	10	6,762,405	90,316,754	1,437	246,435
11F28	F28	11	6,752,681	86,885,619	1,304	196,830
12F28	F28	12	6,758,397	94,661,520	1,836	371,293
13F28	F28	13	6,761,382	83,573,595	1,079	348,663
14F28	F28	14	6,763,456	65,101,671	1,895	323,984
15F28	F28	15	6,761,477	88,632,315	1,130	466,403
16F28	F28	16	6,762,948	88,755,813	1,535	132,163
1S0	S0	1	6,758,328	127,951,521	1,953	130,772
2S0	S0	2	6,764,123	82,944,508	1,365	359,559
3S0	S0	3	6,764,278	89,974,540	1,735	196,149
4S0	S0	4	6,764,413	84,031,154	2,052	307,229
5S0	S0	5	6,764,550	88,782,629	2,055	377,347
6S0	S0	6	6,761,458	97,078,021	6,156	339,172
7S0	S0	7	6,764,089	89,195,794	5,526	342,387
8S0	S0	8	6,763,099	67,389,380	1,527	281,563
9S0	S0	9	6,759,455	58,761,694	6,742	498,810
10S0	S0	10	6,762,994	96,063,090	2,056	531,979
11S0	S0	11	6,757,354	104,894,532	1,524	323,515
12S0	S0	12	6,760,741	91,735,170	2,552	666,167
13S0	S0	13	6,763,139	90,366,798	1,240	265,292
14S0	S0	14	6,763,483	65,994,504	2,451	290,496
15S0	S0	15	6,763,132	101,206,424	1,001	370,298
16S0	S0	16	6,763,931	99,210,538	1,581	343,845
1S1	S1	1	6,758,715	126,658,874	2,185	99,029
2S1	S1	2	6,764,109	84,433,091	1,348	436,834
3S1	S1	3	6,764,240	92,764,089	1,643	232,534
4S1	S1	4	6,764,437	78,793,356	2,003	306,384
5S1	S1	5	6,764,548	88,992,920	1,941	464,483
6S1	S1	6	6,761,625	99,661,288	4,882	266,711
7S1	S1	7	6,764,116	89,505,551	4,607	201,071
8S1	S1	8	6,763,263	68,459,681	1,481	326,852
9S1	S1	9	6,759,523	61,776,685	9,132	423,095
10S1	S1	10	6,763,174	95,518,424	1,962	302,893
11S1	S1	11	6,757,931	104,591,990	1,574	255,349
12S1	S1	12	6,762,914	92,535,338	2,346	461,659
13S1	S1	13	6,762,898	94,660,197	1,302	401,408

Supplemental Table S3 cont'd

14S1	S1	14	6,763,761	62,876,815	1,878	306,861
15S1	S1	15	6,763,258	102,708,875	988	611,412
16S1	S1	16	6,763,898	100,542,883	1,469	324,109
1S28	S28	1	6,756,987	123,963,729	2,184	347,814
2S28	S28	2	6,763,911	80,578,318	1,642	143,603
3S28	S28	3	6,764,212	88,139,626	1,902	306,150
4S28	S28	4	6,764,405	74,676,856	3,144	272,104
5S28	S28	5	6,764,535	94,106,050	1,644	339,358
6S28	S28	6	6,761,578	94,357,330	6,109	654,454
7S28	S28	7	6,762,071	85,350,240	3,286	364,574
8S28	S28	8	6,763,042	79,355,842	1,040	347,205
9S28	S28	9	6,760,522	61,010,633	11,366	102,166
10S28	S28	10	6,762,842	99,013,378	1,608	384,385
11S28	S28	11	6,758,972	100,609,435	1,632	278,698
12S28	S28	12	6,761,152	107,649,527	1,373	343,194
13S28	S28	13	6,762,983	94,188,864	1,333	379,193
14S28	S28	14	6,763,853	65,471,122	1,657	305,052
15S28	S28	15	6,763,363	101,051,271	969	492,302
16S28	S28	16	6,763,676	97,865,044	1,699	282,599

Sequencing Assembly Metrics. Sequencing assembly metrics were tabulated for each sample after normalization of number of non-human passed reads.

Supplemental Table S4. Sequencing metrics: Wilcoxon Rank Sum analysis results		
Metric	Comparison	p-value
% Human reads	Basal vs Frozen	0.429374065
% Human reads	Basal vs Stabilized	0.067693846
% Human reads	Frozen vs Stabilized	0.005764041
% Bacterial reads	Basal vs Frozen	0.208298271
% Bacterial reads	Basal vs Stabilized	0.693106065
% Bacterial reads	Frozen vs Stabilized	0.239744887
% Archaea reads	Basal vs Frozen	0.916121258
% Archaea reads	Basal vs Stabilized	0.342521075
% Archaea reads	Frozen vs Stabilized	0.84646764
% Eukaryota reads	Basal vs Frozen	0.704176543
% Eukaryota reads	Basal vs Stabilized	0.543303716
% Eukaryota reads	Frozen vs Stabilized	0.764954314
% Virus reads	Basal vs Frozen	0.629124013
% Virus reads	Basal vs Stabilized	0.777214236
% Virus reads	Frozen vs Stabilized	0.950003308
Low quality reads	Basal vs Frozen	0.316201118
Low quality reads	Basal vs Stabilized	0.290768656
Low quality reads	Frozen vs Stabilized	0.034736098
Duplicated reads	Basal vs Frozen	0.188368101
Duplicated reads	Basal vs Stabilized	0.029644929
Duplicated reads	Frozen vs Stabilized	0.001394051
Contig length	Basal vs Frozen	0.32903273
Contig length	Basal vs Stabilized	0.271710278
Contig length	Frozen vs Stabilized	0.098499076
Longest contig	Basal vs Frozen	0.359364398
Longest contig	Basal vs Stabilized	0.47130121
Longest contig	Frozen vs Stabilized	0.341523888

Statistical analysis of Sequencing Assembly Metrics.

Wilcoxon Ranked Sum test was used to generating *p*-values for pair-wise comparisons of sequencing assembly metrics across sample types, using all samples.

Supplemental Table S5. Phyla Fold change relative to Basal

Phyla	F0 Fold Change	F1 Fold Change	F28 Fold Change	S0 Fold Change	S1 Fold Change	S28 Fold Change	F0 vs S0 p-value	F1 vs S1 p-value	F28 vs S28 p-value			
Firmicutes	-0.01	-0.62	0.04	0.09	0.15	0.21	0.6772	0.03864	0.0155			
Firmicutes	0.04	0.07	-0.30	-0.04	0.00	-0.02						
Firmicutes	-0.12	0.21	-0.12	0.08	0.08	0.07						
Firmicutes	-0.14	-0.42	-0.40	0.14	-0.13	0.08						
Firmicutes	-0.26	-0.33	-0.39	-0.28	-0.21	-0.08						
Firmicutes	-0.16	-0.07	-0.28	-0.10	-0.01	-0.07						
Firmicutes	0.23	0.31	0.52	0.40	0.40	0.29						
Firmicutes	-0.26	0.05	-0.03	0.33	0.54	0.60						
Firmicutes	-0.09	-0.06	0.16	-0.14	0.16	0.18						
Firmicutes	0.04	0.00	-0.12	0.15	0.10	0.25						
Firmicutes	-0.38	0.17	-0.19	0.29	0.32	0.23						
Firmicutes	0.03	0.49	0.32	0.30	0.38	0.45						
Firmicutes	0.07	-0.03	0.90	1.26	1.26	1.36						
Firmicutes	0.06	0.89	0.65	0.38	0.25	-0.06						
Firmicutes	0.01	-0.07	0.11	0.26	0.31	0.35						
Firmicutes	0.05	-0.26	-0.31	0.33	0.27	0.30						
Bacteroidetes	0.00	0.57	-0.07	-0.13	-0.21	-0.31				0.06396	0.4697	0.01825
Bacteroidetes	-0.03	-0.06	0.16	0.03	0.00	0.02						
Bacteroidetes	0.06	-0.21	0.07	-0.06	-0.07	-0.05						
Bacteroidetes	0.05	0.12	0.11	-0.04	0.05	0.00						
Bacteroidetes	0.11	0.14	0.15	0.12	0.10	0.05						
Bacteroidetes	0.09	0.05	0.13	0.06	0.02	0.06						
Bacteroidetes	-0.14	-0.20	-0.36	-0.27	-0.27	-0.17						
Bacteroidetes	0.09	-0.01	0.02	-0.14	-0.26	-0.30						
Bacteroidetes	0.01	-0.06	-0.08	0.06	-0.01	-0.11						
Bacteroidetes	-0.02	0.00	0.06	-0.08	-0.05	-0.14						
Bacteroidetes	0.14	-0.10	0.08	-0.15	-0.17	-0.11						
Bacteroidetes	0.06	-0.42	-0.16	-0.07	-0.14	-0.17						
Bacteroidetes	-0.02	0.01	-0.37	-0.63	-0.64	-0.73						
Bacteroidetes	-0.02	-0.60	-0.39	-0.17	-0.11	0.02						
Bacteroidetes	0.00	0.18	0.02	-0.28	-0.35	-0.40						
Bacteroidetes	-0.03	0.11	0.14	-0.21	-0.16	-0.18						

Phyla Fold Change relative to Basal. A list of the fold changes of Bacteroidetes and Firmicutes phyla relative to Basal for Frozen (F) and Stabilized (S) samples across the time course. F0, F1, F28: Frozen Day 0, Day 1, and Day 28; S0, S1, S28: Stabilized Day 0, Day 1, and Day 28.

Supplemental Table S6. Richness and SDI scores and statistical analysis results

Timepoint	Subject	Basal SDI	Basal richness	Frozen SDI	Frozen richness	Stabilized SDI	Stabilized richness
Day 0	1	4.49	309	4.53	315	4.59	321
Day 0	2	4.09	280	4.12	278	4.10	282
Day 0	3	4.14	266	4.14	260	4.15	265
Day 0	4	4.11	239	4.06	233	4.15	237
Day 0	5	3.96	245	3.84	232	3.88	243
Day 0	6	4.18	258	4.10	249	4.19	251
Day 0	7	3.92	218	4.03	237	4.06	238
Day 0	8	3.41	284	3.19	271	3.43	280
Day 0	9	3.05	191	3.00	189	3.02	184
Day 0	10	4.13	264	4.14	265	4.21	274
Day 0	11	4.02	273	3.85	261	4.15	274
Day 0	12	3.89	243	3.86	238	3.93	261
Day 0	13	3.10	250	3.19	257	3.92	294
Day 0	14	3.62	229	3.62	219	3.75	239
Day 0	15	4.16	302	4.15	302	4.23	307
Day 0	16	4.19	284	4.21	285	4.30	296
Day 1	1			4.44	306	4.54	318
Day 1	2			4.25	302	4.15	286
Day 1	3			4.35	290	4.21	273
Day 1	4			3.99	228	4.04	231
Day 1	5			3.96	243	3.88	242
Day 1	6			4.11	257	4.21	257
Day 1	7			4.08	256	4.07	243
Day 1	8			3.38	285	3.47	271
Day 1	9			2.98	194	3.12	192
Day 1	10			4.13	265	4.20	270
Day 1	11			4.13	281	4.16	272
Day 1	12			4.00	262	3.95	260
Day 1	13			3.14	258	4.00	303
Day 1	14			3.82	254	3.71	237
Day 1	15			4.12	300	4.24	307
Day 1	16			4.11	272	4.30	295
Day 28	1			4.47	311	4.54	314
Day 28	2			3.99	274	4.08	278
Day 28	3			4.01	257	4.11	268
Day 28	4			3.98	229	3.93	220
Day 28	5			3.86	232	3.81	238
Day 28	6			4.09	248	4.11	245
Day 28	7			4.01	242	4.07	245
Day 28	8			3.30	286	3.54	277
Day 28	9			3.12	205	3.00	182
Day 28	10			4.11	265	4.19	272
Day 28	11			3.88	261	4.12	268
Day 28	12			3.97	252	4.26	297
Day 28	13			3.78	290	3.95	301
Day 28	14			3.76	243	3.68	224
Day 28	15			4.09	295	4.20	308
Day 28	16			4.07	275	4.29	296
Metric	Comparison	Statistical Test		p-value			
Diversity	Frozen vs Time	1-way ANOVA		0.551504762			
Diversity	Stabilized vs Time	1-way ANOVA		0.968167644			
Richness	Frozen vs Time	1-way ANOVA		0.103835023			
Richness	Stabilized vs Time	1-way ANOVA		0.96475062			
Richness	Frozen vs Stabilized	Rank Sum		0.106944848			
Diversity	Frozen vs Stabilized	Rank Sum		0.001711375			

Statistical analysis of Diversity. SDI and richness scores are listed for each sample at each timepoint and treatment. 1-way ANOVA or Wilcoxon Rank Sum tests, as listed, were used to generate p-values comparing richness and diversity scores across sample types.